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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,390,290**, on June 11, 2002, by **PANAGIN PHARMACEUTICALS INC.**, assignee of Dong Huang, for "Protopanaxadiol and Protopanaxatriol and Their Use as Anti-Cancer Agents".

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ABSTRACT

The invention provides for the use of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol in synergistic combination with another cancer chemotherapeutic in pharmaceutical compositions used in methods of inhibiting the multiplication of cancer cells, and methods of treating cancer in patients,comprising administering to such patients therapeutically and synergisticallyeffective amounts of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol in combination with a chemotherapeutic selected from the group consisting of paclitaxel and mitoxantrone. The cancer cells to be treated may be multi-drug resistant. The cancer cells may, for example, be prostate cancer cells or breast cancer cells.

PROTOPANAXADIOL AND PROTOPANAXATRIOL AND THEIR USE AS ANTI-CANCER AGENTS

FIELD OF THE INVENTION

[0001] This invention relates to a novel combination of sapogenin protopanaxadiol and/or protopanaxatriol and other anti-cancer agents, and the use of this combination in cancer treatment applications.

BACKGROUND OF INVENTION

[0002] Since the beginning of the last decade, anti-cancer research has been increasingly directed to the discovery of novel anti-cancer agents obtained from natural sources, as well as identifying and preparing synthetic compounds found in natural sources.

[0003] Ginseng saponins (dammarane saponins, also called "ginsenosides", which are effective ingredients that organically exist in panax ginseng, panax quinguefol, panax notoginseng and other species in the ginseng family) and sapogenins (those that do not naturally exist in the ginseng plant or other species in the ginseng family and can be derived only through chemical structure modification by cleavage and/or semi-synthesis of dammarane saponins), as natural-source root compounds, have been broadly researched for their anti-cancer characteristics. Some of them have been reported to have anti-cancer effects, of which, for example, ginsenoside Rh2

[3-O-β-D-glucopyranosyl-20(s)- protopanaxadiol] has been reported for its anti-cancer activities [I], including induction of differentiation and apoptosis in cancer cells [5~11], inhibition of the growth of human ovarian cancer in nude mice after oral administration [9], and the ability to inhibit the multiplication of multi-drug resistance (MDR) cancer cells while used with other chemotherapy drugs in vitro [12].

[0004] Ginsenoside Rg3 [3-O-[β-D-glucopyranosyl(l→2)- β-D-glucopyranosyl]-20(s)-protopanaxadiol] has been reported to inhibit the invasion by various cancer cells [13] and suppress the proliferation of human prostate cancer cells [14] in vitro, and to inhibit lung metastasis in mice [15] and peritoneal metastasis in rats [16].

[0005] A metabolite of ginseng saponin produced by human intestinal bacteria. Mc [20-O-[α-L-arabinofuranosyl (l→6)-β-D-glucopyranosyl]-20(s)-protopanaxadiol],

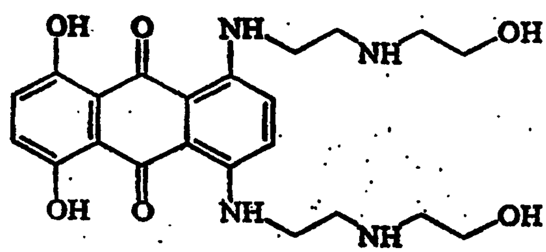
has been reported to inhibit the vascularization of tumors and extravasation of cancer cells [17].

[0006] While conventional chemotherapy agents directly attack the cancer cells and exhibit severe adverse side effects, some ginseng saponins and sapogenins, as well as their intestinal bacteria metabolites, have been reported to have inhibitory effects on cancers by induction of cancer-cell apoptosis and /or by suppression of vascularization of cancers with few adverse side effects.

[0007] In the case of treatment of cancers with ginseng saponins, it has been

reported that saponins which are metabolized to sapogenins by intestinal bacteria have anti-cancer effects.

[0008] Mitoxantrone is an antineoplastic agent which may be prepared as a synthetic antracenedione derivative of the anthraquinone dye ametantrone. Mitoxantrone has the following formula:

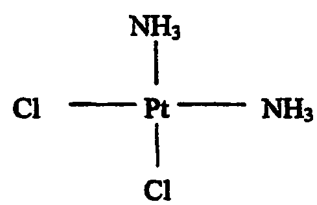


Mitoxantrone has reportedly been used in the treatment of a variety of malignant diseases, including acute non-lymphocytic leukemia, advanced breast cancer and prostate cancer (see Wiseman and Spencer, Drugs & Aging, 1997 June 10(6):473). Mitoxantrone is available commercially, and its preparation is, for example, described in U.S. Patent No. 4,197,249.

[0009] Paclitaxel is a derivatized diterpenoid which may be obtained from the bark of the Pacific Yew and other natural sources (Taxus brevifolia, see Wani et al., J. Am. Chem. Soc.93 :2325, 1971; and Stierle et al., Science 60:214-216, 1993). Therapeutically, particularly in cancer therapy, paclitaxel is thought to act to stabilize microtubular structures by binding tubulin. As used herein, the word "paclitaxel" may include analogues, derivatives and conjugates of the naturally-occurring molecule, such as TAXOL™, TAXOTERE™, 10-desacetyl analogues of paclitaxel, 3'N-desbenzoyl-3'N-t-butoxy carbonyl analogues of paclitaxel, paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylos. Paclitaxel may be prepared utilizing a variety of techniques (see WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076, U.S. Pat. Nos. 5,294,637, 5,283,253, 5,279,949, 5,274,137, 5,202,448,

5,200,534, 5,229,529, and EP 590267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo.

[0010] Cisplatin is an inorganic compound possessing a platinum element.



[0011] Cisplatin (cis-diaininedichloroplatinum (II)) has been used as a chemotherapeutic agent for many years since discovery of its anti-tumor activity (Rosenberg et al, Nature, 205:698, 1965; Nature 222:385, 1972; U.S. Patent No. 4,177,263). The mechanism of action of cisplatin in cancer therapy is believed to be through its ability to bind to DNA to interfere with repair mechanisms, causing cell death. Cisplatin has been reported to be effective in the treatment of a variety of cancers, most significantly in the treatment of ovarian and testicular cancer. Cisplatin is available commercially, for example, from Bristol-Myers Squibb under the commercial name PLATINOL™.

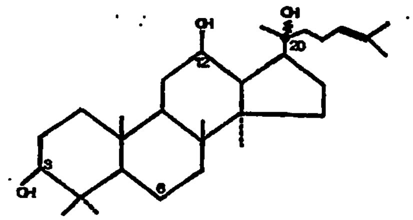
[0012] Cancerous tumors that have responded well initially to a particular drug or drugs, may later develop a tolerance to the drug(s) and cease responding, a phenomenon known as multi-drug resistance. Multi-drug resistance is generally characterized by cross-resistance of a disease such as cancer to more than one functionally or structurally unrelated drugs. Multi-drug resistance may be caused by a number of mechanisms. For example, multi-drug resistance may be mediated by a protein that is variously called multi-drug-resistance 1 protein (MDR1),

pleiotropic-glycoprotein (P-glycoprotein), Pgp or P170, referred to herein as "P-glycoprotein". P-glycoprotein is thought to be endogenous in cell membranes in certain drug resistant cells, multi-drug resistant tumor cells, gastrointestinal tract cells, and the endothelial cells that form the blood brain barrier, where P-glycoprotein is thought to act as an efflux pump for the cell. Certain substances, including treatment drugs for various diseases, may be pumped out of the cell by the P-glycoprotein prior to having a therapeutic effect on the cell. There is accordingly a need for therapeutic approaches that may be used to counteract drug resistance, particularly multi-drug resistance mediated by P-glycoprotein in cancer.

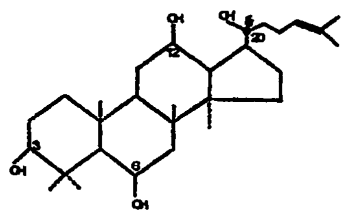
SUMMARY OF THE INVENTION

[0013] In one aspect, the invention provides methods inhibiting the multiplication of cancer cells, and methods of treating cancer in patients in need of such treatment, comprising administering to such patients therapeutically and synergistically effective amounts of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol in combination with a chemotherapeutic selected from the group consisting of paclitaxel and mitoxantrone. The cancer cells to be treated may be multi-drug resistant. The cancer cells may for example be prostate cancer cells or breast cancer cells.

[0014] The chemical structures of protopanaxadiol and protopanaxatriol are as follows:



Sapogenin Protopanaxadiol (PPD)



Sapogenin Protopanaxatriol (PPT)

[0015] In alternative aspects, the invention provides for the use of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol in combination with a chemotherapeutic selected from the group consisting of paclitaxel and mitoxantrone to synergistically inhibit the multiplication of cancer cells, or to formulate a medicament for inhibiting the multiplication of cancer cells synergistically. The cancer cells to be treated may be multi-drug resistant. The cancer cells may, for example, be prostate cancer cells or breast cancer cells.

[0016] In alternative aspects, the invention provides for the use of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol to render non-P-glycoprotein multi-drug resistant cancer cells (cancer cells that do not express P-glycoprotein) sensitive to a chemotherapeutic. In such embodiments, a chemotherapeutic may, for example, be used with sapogenin protopanaxadiol and sapogenin protopanaxatriol to treat a patient at a concentration or dosage at which the chemotherapeutic alone would otherwise not be effective in said non-P-glycoprotein multi-drug

resistant cancer cells. The chemotherapeutic may, for example, be paclitaxel, mitoxantrone, or cisplatin. The cancer cells may, for example, be prostate cancer cells or breast cancer cells.

[0017] In alternative aspects, the invention provides a pharmaceutical composition for the treatment of cancer, in patients in need of such treatment, comprising a pharmaceutically acceptable carrier and therapeutically and synergistically effective amounts of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol and a chemotherapeutic selected from the group consisting of paclitaxel and mitoxantrone.

[0018] The invention is directed to a method of inhibiting the multiplication of cancer cells by treating the cells with a therapeutically and synergistically effective combination of paclitaxel, and sapogenin protopanaxadiol and/or sapogenin protopanaxatriol.

[0019] The invention also pertains to a method of inhibiting the multiplication of cancer cells by treating the cells with a therapeutically and synergistically effective combination of mitoxantrone, and sapogenin protopanaxadiol and/or sapogenin protopanaxatriol.

[0020] The cancer cells can be multi-drug resistant and need not express P-glycoprotein. The cancer cells can be selected from the group consisting of prostate cancer cells and breast cancer cells.

[0021] The invention also includes a method of treating a patient having a cancer with a therapeutically and synergistically effective combination of paclitaxel and sapogenin protopanaxadiol and/or sapogenin protopanaxatriol, and a method of treating a patient having a cancer with a therapeutically and synergistically effective combination of mitoxantrone, sapogenin protopanaxadiol and/or sapogenin protopanaxatriol.

[0022] The invention includes the use of a therapeutically effective amount of sapogenin 20(R) protopanaxadiol and sapogenin 20(S) protopanaxatriol in combination with paclitaxel or in combination with mitoxantrone to synergistically inhibit the multiplication of cancer cells.

[0023] The invention also includes the use of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol to formulate a medicament for inhibiting the multiplication of cancer cells synergistically with paclitaxel or mitoxantrone.

[0024] The invention is also directed to a method of inhibiting the multiplication of multi-drug resistant cancer cells, wherein the cells do not express P-glycoprotein, comprising treating the cells with a therapeutically and synergistically effective combination of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol and a chemotherapeutic. The chemotherapeutic can be paclitaxel, mitoxantrone or cisplatin. The multi-drug resistant cancer cells can be selected from the group consisting of prostate cancer cells and breast cancer cells.

[0025] The invention also pertains to the use of a therapeutically effective amount of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol in combination with a chemotherapeutic to inhibit the multiplication of multi-drug resistant cancer cells, wherein the cells do not express P-glycoprotein, and the chemotherapeutic alone is not therapeutically effective to inhibit the multiplication of the cancer cells. The chemotherapeutic can be paclitaxel or mitoxantrone or cisplatin. The multi-drug resistant cancer cells can be selected from the group consisting of prostate cancer cells and breast cancer cells.

[0026] The invention is also directed to a pharmaceutical composition comprising a pharmaceutically acceptable carrier, and therapeutically and synergistically effective amounts of

sapogenin protopanaxadiol and/or sapogenin protopanaxatriol, and a chemotherapeutic selected from the group consisting of paclitaxel and mitoxantrone.

[0027] The form of the composition can be selected from the group consisting of an

orally administrable form, an injectable form, and an externally applicable form. The composition can be the orally administrable form and selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a pill, a liquid, a spirit, a syrup or a limonade, or it can be the injectable form and selected from the group consisting of a liquid, a suspension and a solution. The composition can also be the externally applicable form selected from the group consisting of an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

DRAWINGS

[0028] In drawings which illustrate specific embodiments of the invention, but which should not be construed as restricting the spirit or scope of the invention in any way:

[0029] Figure 1 is a graph illustrating a comparison of cytotoxicity of sapogenin protopanaxadiol (PPD), sapogenin protopanaxatriol (PPT) and Rh2, showing cellular viability of B16 melanoma tumor cells on the vertical axis and the chemotherapeutic concentration on the horizontal axis.

[0030] Figures 2a and 2b are two graphs, A and B, showing the use of sapogenin protopanaxadiol (PPD) and sapogenin protopanaxatriol (PPT) in combination with paclitaxel on breast cancer cells MCF7/MCF7r and showing cellular viability on the vertical axis and paclitaxel concentration on the horizontal axis, with four lines on each graph representing paclitexal only, and paclitexal with Rh2, PPD and PPT.

DETAILED DESCRIPTION OF THE INVENTION

[0031] Throughout the following description, specific details are set forth in order to provide a more thorough understanding of the invention. However, the invention may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

[0032] Sapogenins do not exist in ginseng. Saponins which exist in ginseng must be converted by chemical reaction into sapogenins. Cancers susceptible to treatment with sapogenin protopanaxadiol and sapogenin protopanaxatriol in combination with a chemotherapeutic 25 in accordance with various aspects of the invention may include both primary and metastatic tumors and hyperplasias, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract, (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma) and tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas). In some aspects of the invention, sapogenin protopanaxadiol and sapogenin protopanaxatriol in combination with a chemotbrapeutic may also be useful in treating hematopoietic cancers such as leukemias (i.e. chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphomal leukemia) and lymphomas (both Hodgkin's and non-Hodgkin's

lymphomas). In addition, sapogenin protopanaxadiol and sapogenin protopanaxatriol in combination with a chemothrapeutic may be useful in the prophylactic prevention of metastases from the tumors described above.

[0033] Sapogenin protopanaxadiol and/or sapogenin protopanaxatriol and the chemotherapeutic may be administered in combination separately or as one single combined pharmaceutical composition. The amount of each component administered may be determined by an attending clinician, taking into consideration a variety of factors such as the etiology and severity of the disease, the patient's condition and age and the potency of each component. The components may be administered in accordance with the standard methodologies as for example disclosed in the Physician's Desk Reference (PDR) published by Medical Economics Co. Inc. of Oradell, N.J.

[0034] A therapeutically and synergistically effective combination of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol and a chemotherapeutic is characterized by the fact that the chemotheraputic is administered at a chemotherapeutic dosage and sapogenin protopanaxadiol and/or sapogenin protopanaxatriol is administered at a therapeutic dosage, and the therapeutic effect thereby achieved, such as inhibition of cellular multiplication, is greater than the sum of the therapeutic effect that would be achieved with the chemotherapeutic alone at the chemotherapeutic dosage plus the therapeutic effect that would be achieved with sapogenin protopanaxadiol and/or sapogenin protopanaxatriol alone at the therapeutic dosage. For example, a therapeutically and synergistically effective combination of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol and paclitexal is a combination wherein the paclitexal is administered at a paclitexal dosage and sapogenin protopanaxadiol and/or sapogenin protopanaxatriol is administered at a therapeutic dosage, and the inhibition of cellular multiplication thereby achieved is greater than the sum of the inhibition that would be achieved

with paclitexal alone at the paclitexal dosage plus the inhibition that would be achieved with sapogenin protopanaxadiol and/or sapogenin protopanaxatriol alone at the therapeutic dosage. Similarly, a therapeutically and synergistically effective combination of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol and mitoxantrone is a combination wherein the mitoxantrone is administered at a mitoxantrone dosage and sapogenin protopanaxadiol and/or sapogenin protopanaxatriol is administered at a therapeutic dosage, and the inhibition of cancer cell multiplication thereby achieved is greater than the sum of the inhibition that would be achieved with mitoxantrone alone at the mitoxantrone dosage plus the inhibition that would be achieved with sapogenin protopanaxadiol and/or sapogenin protopanaxatriol alone at the therapeutic dosage.

[0035] One or more pharmaceutically acceptable carriers or exipients may be used to formulate pharmaceutical compositions of the invention, including solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In alternative embodiments, the carrier may be suitable for parenteral, intravenous, intraperitoneal, intramuscular, sublingual or oral administration. Pharmaceutically acceptable carriers may include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the pharmaceutical compositions.

[0036] Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a

solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use ofsurfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the pharmaceutical compositions may be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglyeolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglyeolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

[0037] Sterile injectable solutions can be prepared by incorporating an active compound in the required amount in an appropriate solvent with one or a

combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which

yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Pharmaceutical compositions may be formulated with one or more compounds that enhance the solubility of the active compounds.

[0038] Procedures for the isolation and purification of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol may for example include aqueous or organic extraction, column chromatography, thin-layer chromatography, and high performance chromatography. Techniques for the extraction and purification of plant extracts may be adapted for the preparation of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol from the root of *Panax ginseng*, such as techniques disclosed in the following documents (which are incorporated herein by reference): U.S. Patent No. 6,156,291 issued to Pang, et al. on December 5, 2000; U.S. Patent No. 6,083,932 issued to Pang, et al. on July 4, 2000; U.S. Patent No. 4,157,894 issued to Bombardelli on June 12, 1979; U.S. Patent No. 5,137,878 issued to Pang, et al. on August 11, 1992; U.S. Patent No. 5,230,889 issued to Inoue on July 27, 1993; U.S. Patent No. 5,589,182 issued to Tashiro, et al. on December 31, 1996.

[0039] Figure 1 shows that PPD or PPT has more potent tumor inhibitory effect than Rh2. The cytotoxicity of protopanaxadiol (PPD), or protopanaxatrial (PPT), and Rh2 was compared in B16 melanoma tumor cells. Cells were treated with various concentrations of the compounds and the viability was measured 24 hours post treatment.

[0040] Figures 2a and 2b show enhanced cytotoxicity of paclitexal on drug sensitive (MCF7) or drug resistant (MCF7r) human breast cancer cells in the presence of sapogenin protopanaxadiol or sapogenin protopanaxatriol. Cells were either treated with various concentrations of paclitexal alone or in the presence of 20 /xg/ml Rh2, 6/xg/ml PPT or PPD. All three of the compounds

synergistically enhanced taxol induced cytotoxicity. But PPD is significantly more potent in synergy with taxol. This synergy was most prominent in drug resistant tumor cells (MDF7r).

[0041] The following table illustrates enhancement effect of Rh2, sapogenin protopanaxadiol and sapogenin protopanaxatriol on IC50 of taxol on drug sensitive (MCF7) and drug resistant (MCF7r) cell lines. It should be noted that the enhancement effect of all three compounds was much more dramatic in drug resistant cell lines than in the drug sensitive cells, especially PPD and PPT.

Table 1

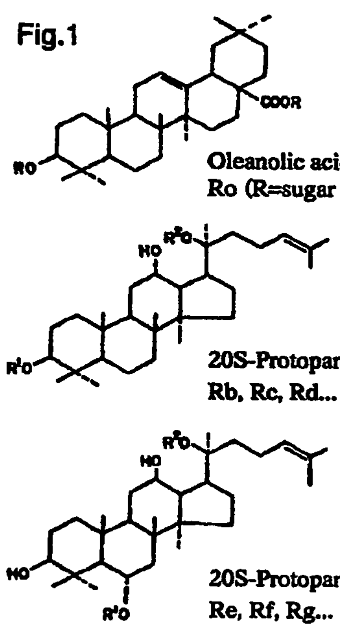
Cell Line	Taxol only	Rh2 (20 μg/ml)	Protopanaxadiol (6 μg/ml)	Protopanaxatrial (6 μg/ml)
MCF7	225 nM	61.6nM (3.65 fold)	12.5nM (18 fold)	45.8 nM (4.9 fold)
MCF7r	929.8 nM	0.208 nM (4470 fold)	0.0315 nM (29517 fold)	0.533 nM (1744 fold)

1. General Background

Many cancer patients who undergo conventional treatment also supplement their medical regimens with complementary therapies. It has been reported in the United States that Americans made 425 million visits to alternative practitioners compared with 388 million visits to conventional doctors' offices in 1990 [1]. It was estimated that the sale of herbal medicines reached 2 billion in 1996 and more than 63% of population would be using herbs as part of their daily routine by 2001 [2-4]. The need to expand our knowledge regarding many naturopathic treatments is therefore of great importance. The benefit of the investigation of this nature would include leads of novel therapeutic agents, reducing unsafe uses of herbal products, and discovery of mode of actions, and so on.

Amongst the Chinese herbal medicines that are widely used in the world, Panax ginseng has attracted a great deal of attention. Ginseng has long been recognized as a general tonic—good for recovery from exhaustion, surgery, and energy loss and for treating debilitating effects of old-age. Ginseng is also established as a benign and safe herb. In recent years, the beneficial effects of ginseng in the treatment of diseases such as cancer, diabetes mellitus and arteriosclerosis have been reported (see review[5, 6]).

Fig.1



Oleanolic acid (R=H)
Ro (R=sugar moiety)

20S-Protopanaxadiol (R^1=R^2=H)
Rb, Rc, Rd... (R^1=R^2=sugar moiety)

20S-Protopanaxatrial (R^1=R^2=H)
Re, Rf, Rg... (R^1=R^2=sugar moiety)

As early as in 1854, a saponin fraction was isolated from American ginseng, Panax quinquefolium L. by Garriques of the USA, and was named "panaquilon". It was in late 1950s, isolating and characterizing saponin fractions, collectively called *ginsenosides*, was achieved from Korean ginseng by the Japanese scientists Drs. S. Shibata and O.Tanaka. Their work is a milestone in the advancement of ginseng research[7, 8]. Several classes of components have since been isolated and they include: ginsenosides, carbohydrates, nitrogenous compounds, fat-soluble compounds, vitamins and minerals. It is believed that ginsenosides are the main active components of ginseng.

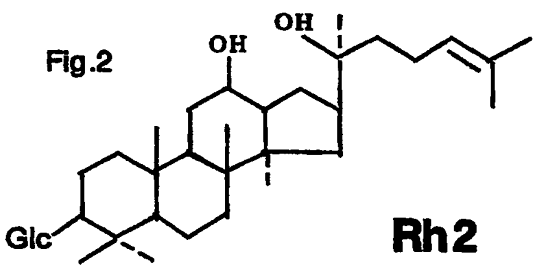
Ginsenosides are composed of a sugar portion (glycon) and a non-sugar portion (aglycon or sapogenin). Aglycon, the backbone of ginsenosides, is classified into three types: protopanaxadiol, protopanaxatriol, and oleanoic acid. Protopanaxadiol and protopanaxatriol are tetracyclic terpenes of the dammarane series while oleanoic acid is not (Fig.1) The side chain of the sapogenins can be further modified. Idividual, isolated ginsenosides were named according to the order of the Rf value on thin layer chromatograms (for example, Ra, Rb1, Rb2) [5, 9-27]

Numerous studies have been conducted to explore the potential therapeutic components

of ginsenosides and their mechanisms of action[5, 6]. A variety of biological activities have been determined for some compounds including effects on ion channel activity in myocardiocytes and neuronal cells [28-34]; stimulation of DNA, protein and lipid synthesis in various types of cells [35-45], affects on gene expression and post-transcriptional regulation of the stability of mRNAs [36, 38, 46-48]. A wide range of potential therapeutic applications for ginsenosides have been claimed but most of the earlier research has been focused on the protective effects of protopanaxadiol ginsenosides (especially Rb group) on ischemia induced tissue damage in cardiovascular and central nervous systems. This has mainly been due to the large quantity of these compounds which are found in ginseng roots. With advanced technology, more ginsenosides components, especially the protopanaxatriol groups were isolated and studied for their biological activity [19, 25, 40, 49-54]. The best studied compounds in these groups are Rg. It has been reported that Rg has a variety of effects on cells including protecting pulmonary vascular endothelium against free radical induced injury, up-regulating cyclin E-dependent kinase activity in human hepatoma cells; selectively enhancing the proliferation of lymphocytes and the production of IL-2 [32, 38, 44, 55-62].

In the middle of the 1980s, when the Rh group was isolated [24, 26, 54, 63-67], a detailed study was initiated to examine potential anti-cancer effects of ginsenosides. To date, four compounds, Rh1 to Rh4 have been isolated in the Rh group, each differentiated by the position of a glycon. Little is known about the biological activity of the most recently isolated Rh3 and Rh4. However, it was reported that Rh1 and Rh2 could induce differentiation in B16 melanoma and F9 teratocarcinoma stem cells since the middle of 1980s when the Rh group was isolated [68-70]. Rh2 has since been the focus of attention mainly because of its manifested strong tumor inhibitory effect. Besides inducing the differentiation on B16 melanoma cells, it also inhibits the proliferation of murine melanoma, human MCF-7 breast cancer cells, ovarian cancer cells and SK-HEP-1 cells *in vitro* [39, 43, 71, 72 and our results (see below)]. Orally administrated Rh2 could also inhibit the growth of human ovarian cancer in nude mice[73]. In addition, *in vitro* studies have demonstrated the ability of high concentrations of Rh2 to instigate apoptotic cell death in cancer cells [42, 72-74]. The study of the mechanism for the tumor inhibitory effect of Rh2 have recently begun. As a result of these studies, it has been speculated that the growth inhibition effects of Rh2 may involve expression of p27kip1[43], p21WAF1/CIP1[72] and/or suppression of cyclin-dependent kinase-2 (Cdk2) [71]. Rh2 also markedly reduced the phosphorylated retinoblastoma protein (pRb) and enhanced association of unphosphorylated pRb and the transcription factor E2F-1 in MCF7 cells [72]. It has been reported and confirmed by our own results (see below) that apoptosis induced by Rh2 involves caspase 3 but is independent of the Bcl-2 family [42, 74-76].

Fig.2



Rh2

2. Chemical Composition Profile of a Ginsenoside Composition

A ginsenoside composition(s) is a natural product from American ginseng, Panax quinquefolium L. A ginsenoside composition(s) has excluded other ginsenosides from ginseng,

and contains only anti-cancer ginsenosides such as Rh2 and other novel aglycon ginsenosides. Furthermore, the proprietary technologies allow the manufacturer to provide highly purified ginsenosides. Each capsule of a ginsenoside composition contains 100 mg of anti-cancer ginsenosides (>99% purity), an amount equivalent to that extracted from hundreds of pounds of ginseng using conventional methods, but at a cost of less than one thousandth of using ginseng! Comparing to other ginseng products in the market, a ginsenoside composition has the following distinctive features:

1. It is not a general ginseng extract but a complex containing a specific group of ginsenosides with defined anti-cancer activity. Since different groups of ginsenosides have different biological activities and some of them may even be contradictory, the general ginseng extract may have less therapeutic effect for a specific disease. In addition, amounts of ginsenosides with anti-cancer activity such as Rh2 and protopanaxadiol aglycons are extremely low in ginseng (Rh2 is less than 0.04% in total extracted ginsenosides and aglycons are barely detectable), it is impossible to achieve a therapeutic concentration of this group of ginsenosides from the normal ginseng extract. In contrast, 80% of the ginsenosides in the ginsenoside composition(s) are Rh2, protopanaxidial, protopanaxitriol aglycons and other novel sapogenins. Each of them has shown anti-cancer activities in experimental settings (see below). This makes the ginsenoside composition(s) a highly specific anti-cancer agent.

2. Unlike any other nature products, a ginsenoside composition(s) is not only standardized but also has a well defined chemical composition profile. Among the ginsenosides contained in the ginsenoside composition(s), more than 80% of them have been isolated and characterized for their chemical identities.

The components of ginsenosides in the ginsenoside composition(s):

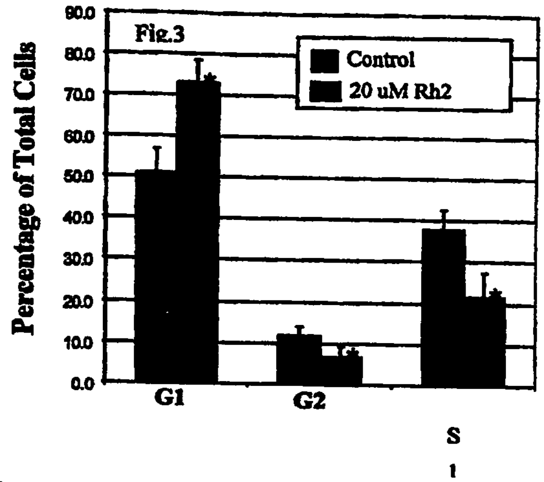
Rh2	8%
Rg3	*2%*
Sapogenins	*70%*
Unknown ginsenosides	*20%*

3. Anti-Cancer Activity Of Ginsenosides In A Ginsenoside Composition(s): A Pharmacological Profile

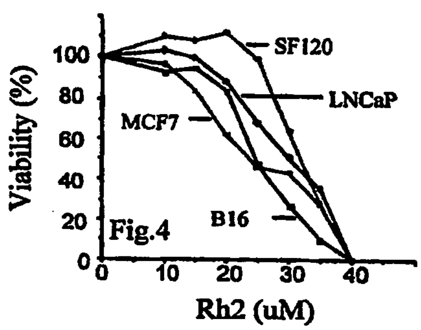
As indicated in the list of ingredients of a ginsenoside composition(s), 80% of the ginsenosides are Rh2, and protopanaxadiol or protopanaxatriol aglycons. As a representative compound of this group of ginsenosides, Rh2 has been studied in the most details.

Low concentrations of Rh2 induced cell-growth arrest

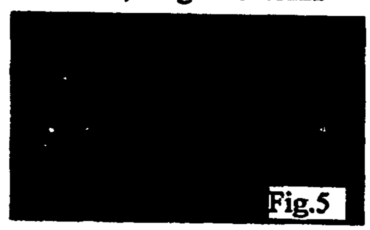
Fig.3 shows the cell-cycle status of B16 cells treated with 20 μM of Rh2 for 24 hours, determined by flow cytometry. Compared to the untreated cells, 20 μM of Rh2 caused 22.3% ($p<0.00001$) increase in number of cells at G1, and 5.3%($p<0.00001$) and 16.3%($p<0.00001$) reduction in number of cells at G2 and S phases, respectively. These results suggest that treatment with Rh2 at this concentration inhibited cell proliferation by arresting cells at the G1 phase of the cell cycle. When B16 cells were treated with similar concentrations (15-20 μM) of Rh2 for longer periods, the cell-growth arrest was more evident and the cells eventually died within 10 days.

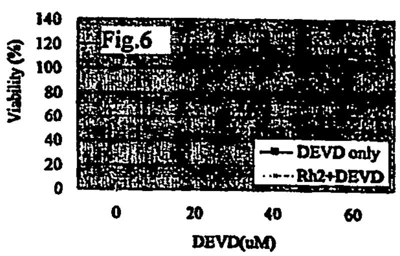


High concentrations of Rh2 caused rapid apoptosis in cancer cells



As shown in Fig. 4, Rh2 exhibited similar cytotoxicity to various cancer cell lines, including human glioblastoma (SF120), prostate cancer (LNCaP), breast cancer (MCF7) and mouse melanoma (B16), with approximate IC50s between 20-30 μM. Similar results were also obtained in human gastric carcinoma cells Kato III, lung carcinoma cells H460 and H838, and lung mesothelioma (data not shown). At concentrations of 40 to 80 μM, Rh2 induced apoptotic cell death within a few hours, evidenced by nuclear fragmentation as shown in Fig.5.



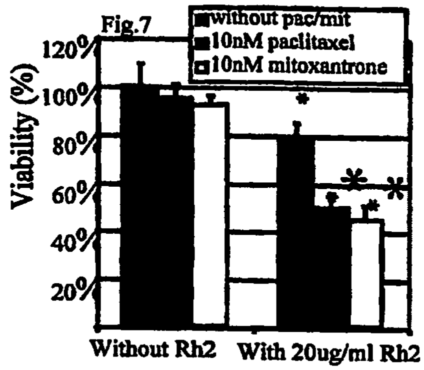


The apoptotic effect of Rh2 was caspase-dependent

As an initial investigation into the mechanism of Rh2-induced apoptosis, an inhibitor for caspases, DEVD, was used. B16 cells were pretreated with 30 μM of Rh2 in the presence of the peptide at various concentrations. As shown in Fig. 6, the cell death induced by Rh2 could be completely blocked with DEVD in a dose-dependent fashion. The calculated IC50 of DEVD was 30 μM.

Rh2 synergistically enhanced the cytotoxic effect of chemotherapeutic agents

Human prostate-cancer cells LNCaP were incubated with 20 μg/ml of Rh2 in the presence of 10 nM of either paclitaxel or mitoxantrone (Figure 7). Remarkable synergy was evident in cells co-treated with Rh2 and paclitaxel or mitoxantrone. 20 μg/ml Rh2 alone had a mild effect on the viability of LNCaP cells compared to that of vehicle-treated controls (78.3±7%, $p<0.05$).



However, the combination of Rh2 and paclitaxel or mitoxantrone significantly enhanced the cytotoxicity, clearly indicating a synergistic effect between the Rh2 and the two chemotherapy agents. Even more strong synergy was observed in drug resistant mesothelioma cells (Fig.8).

Rh2 hypersensitized multidrug-resistant cells to paclitaxel

Human breast-cancer cells MDA435/LCC6 cells, both wild-type (MDA435/LCC6W, L6W) and drug resistant (MDA435/LCC6M, L6M) strains were incubated with 20 or 30 µg/ml of Rh2 in the presence of paclitaxel at various concentrations, and the IIC50s were calculated 24 h after the treatment. As shown in Table 1, the IC50s for drug sensitive cells L6W and the p-gp expressing daughter cell line L6M are 248nM and 631nM, respectively. The resistant index is therefore $IC50_{(L6M)}/IC50_{(L6W)}$ =2.54. With 20 µg of Rh2, the paclitaxel's IC50s for L6W and L6M became 75nM and 0.15nM, respectively, giving the resistant index of 0.002 that is much smaller than 1. The reversed index showed that the p-gp+ cells became 500 folds more sensitive to taxol than the p-gp- parental cells following the Rh2 treatment. We called this phenomenon as ***MDR associated Chemosensitization Enhancement(MACE).***

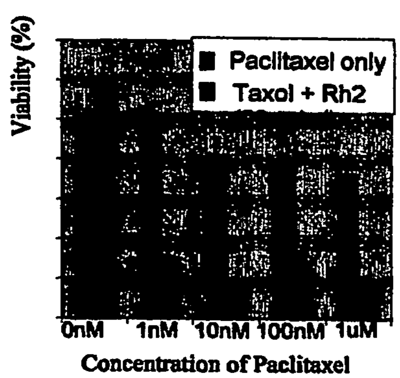


Table 1	Paclitaxel only	Pac+20 μg/ml Rh2	Pac+30 μg/ml Rh2
L6M	631.00 nM	0.15 nM	< 0.03 nM
L6W	248.00 nM	75.00 nM	25.00 nM
Resistant Indices:	2.54	0.002	0.001

Aglycon sapogenins have similar anti-cancer effects as Rh2

70% of ginsenosides in the ginsenoside composition(s) are sapogenins, including protopanaxadiol (PPD), PAM-120 and PBM-100. Fig.9 compares the tumor cell cytotoxicity of Rh2, Rg3 and PPD on B16 melanoma cancer cells. At the same low concentration (6 ug/ml), PPD seems more potent than Rh2 or Rg3 ($p<0.015$). Similarly, the two new sapogenins PAM120 and PBM100 both demonstrated cytotoxicity in drug resistant human breast cancer cells as shown in Fig.10.

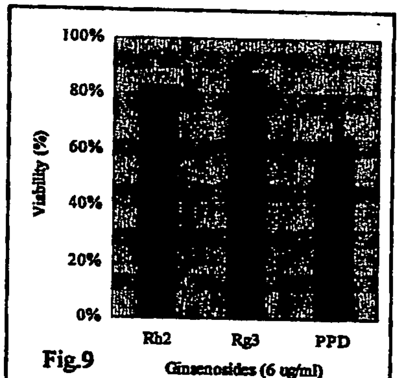
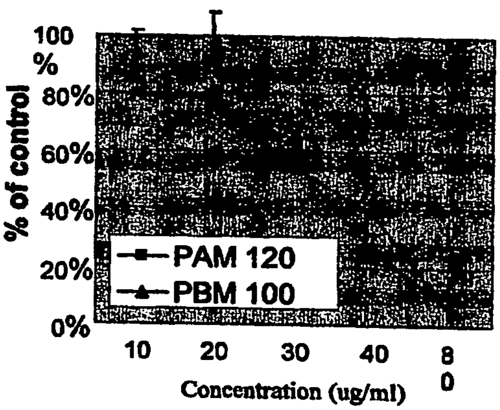
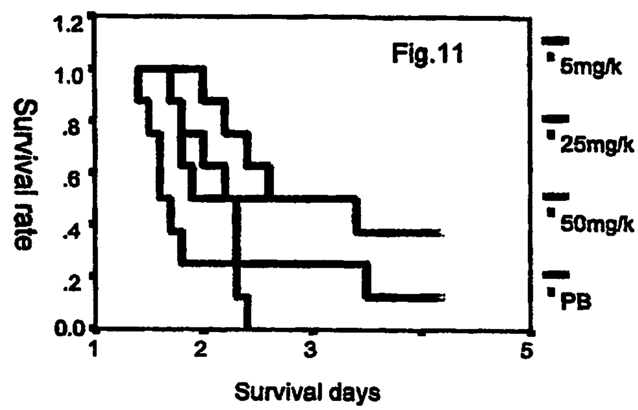


Fig.9



In vivo cancer inhibitory effect of a ginsenoside composition.

To test the anticancer activity of a ginsenoside composition in vivo, an animal malignant brain tumor model was utilized. Tumor cells were implanted into rat brain and three doses of a ginsenoside composition were given orally on day 10 postimplantation when the tumor had been established. As shown in Fig.11, after 2 x5 days ginsenoside composition treatment, there are significant difference in survival rates of animals between the treatment group and control. Especially for animals with 25mg/kg and 50mg/kg, 40% of animals survived longer than 42 days comparing to the average of 18 days in control animals. Furthermore, more than 50% of survived animals were found completed tumor regression by autopsy.



4. Toxicological Profile of A Ginsenoside Composition : No Detectable Toxicity

Formal acute and long term toxicity tests of a ginsenoside composition have been conducted. Briefly, for acute toxicity test, rats were orally given 4000 mg/kg of the ginsenoside composition once. It was found that treated animals appeared quiet and less active with eyes closed 30 min after the treatment. The drowsiness disappeared in 4 hours and the activity, diet and water intake returned to normal. No animal died during 7 observation days and autopsy did not show any abnormality in heart, liver, spleen, lung, kidney, stomach and intestine. For the long term toxicity test, animals were orally given 333.3 mg/kg, 131.0 mg/kg, and 51.5 mg/kg of the ginsenoside composition as high(H), medium (M) and low (L) dosages, respectively. The treatment was daily and lasted 8 weeks plus 2 weeks post-treatment observation period. Transient hypertrophy in the prostate (187%) and ovary(124%) were found in group H animals in 24 hours. Similar hypertrophy in the prostate (156%) was found in group M. However, no hypertrophy was found after 2 weeks. Other minor toxic reactions were only seen in group H, including drowsiness during week 3 and 4 (back to normal thereafter) and less weight gain (no statistic significance) in later stage of the treatment (week 4-9). The blood routine and organ histology examinations did not shown any abnormality in all the three groups.

Acute Toxicity tests of the ginsenoside composition #2 were conducted. The results are briefly summarized as followings.

I. Blood vessel irritation test:

Ginsenoside composition #2 was given i.v. via the ear vein in rabbits (Japanese Big-ear) at the dose of 5ml/kg (1mg/ml) daily for 7 days consecutively. No noticeable changes were seen in the injection region. Microscopic examination on tissue sections of the injection region did not found venous congestion, edema or infiltration of inflammatory cells.

Conclusion: Ginsenoside composition #2 does not cause irritation to the blood vessels.

II. Endotoxin test:

Limulus Amebocyte Lysate (LAL) interference test was performed to determine the levels of endotoxin in ginsenoside composition #2. The minimum detectable level of

endotoxin was 0.125 EU/ml – 0.25EU/ml for the LAL test kits utilized. No interference was detected with ginsenoside composition #2 at the dilutions of 1mg/ml, 0.5 mg/ml, 0.25 mg/ml and 0.125 mg/ml.
Conclusion: The endotoxin level is less than 1 EU in 1 mg of ginsenoside composition #2.

III. Hemolysis test

Rabbit whole blood was used to mix with dilutions of ginsenoside composition #2 and spectrophotometrical absorbance of the supernatant was measured at the wavelength of 545 nm to determine hemolysis. Saline diluted ginsenoside composition #2 (ginsenoside composition #2 : saline =3:1, final concentration: 1mg/ml) caused hemolytic effect was less than 1% (normal value: <5%). However, undiluted ginsenoside composition #2 (4mg/ml) caused minor hemolysis (51%).
Conclusion: No hemolysis effect can be detected when ginsenoside composition #2 is used in 1:3 saline diluted solution.

IV. Allergy test

The allergy test was performed in genie pigs. 0.5ml of ginsenoside composition #2 was given i.p. every second day for 6 days (3 times). In addition, 1 ml ginsenoside composition #2 was given i.v. to the same animal on day 14 and day 21. Typical allergy responses such as coughing, sneezing, skin/hair and respiratory reactions, etc. were monitored in 15 minutes following the injection.
Conclusion: Ginsenoside composition #2 does not cause allergy.

V. LD50 of Ginsenoside composition #2

Five groups of mice were i.v. injected once with 5 different doses of ginsenoside composition #2 (from 83.5mg/kg to 160.0mg/kg). The animals were observed 7days following the injection and the LD50 was determined using Bliss method. Severe toxicity and death were seen in animals with the dosages of 136 mg/kg and 160 mg/kg. Reducing dosages significantly reduced toxicity.
Conclusion: The LD50 of ginsenoside composition #2 i.v. injection is 121.9mg/kg with a 95% confidence range of 105.0-141.6 mg/kg.

5. Clinical Experience With A Ginsenoside Composition

An informal phase I clinical trial was performed in Liaoning Provincial TCM Research Institute. Forty patients of various types of malignant solid tumors who had failed with first line treatment or refused for conventional treatment were enrolled (Fig.12). The 40 patients with average age of 56.1±11.5 yrs were treated with 200 mg/day of a ginsenoside composition orally for average duration of 53.7±25.4 days. The primary objective was to test for the toxicity of a ginsenoside composition measured by the symptoms of nausea,vomitingstomatitis, hair loss, nervous system symptoms and skin abnormality. None of the above symptoms were repored in any patients observed. The toxicity was then concluded as grade 0.

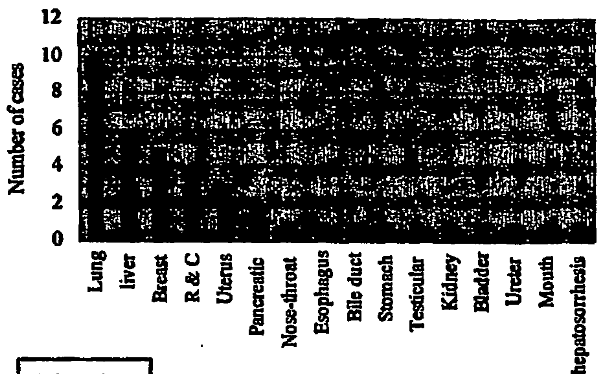


Fig.12

Tumor site

Although it was not the primary goal of this trial, preliminary observations of efficacy were very encouraging. It was reported that 75% of the patients treated with a ginsenoside composition have demonstrated improvement in their symptoms to various degrees and some of them had shown significant tumor regression. The summarized results are shown in Fig.13.

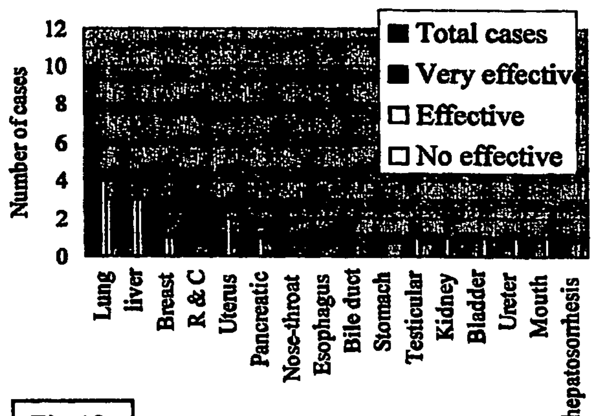


Fig.13

Tumor site

Case Examples:

Example 1:Female, 58 yr, Lower bile duct cancer (3.0x2.6 cm)
Symptom: weight loss, nausea, dizzy, fatigue, upper abdominal pain, icterus, etc.
Treatment: 114 days, 200 mg/day
Improvement: Tumor size reduced to 1.8x1.4 cm, all the above symptoms disappeared, Feeling well by herself.

Example 2: Female, 74 yr, Esophagus cancer (umbrella type), 6.0 cm long (x-ray image)
Symptoms: difficulty in swallow, pain, weight loss, frequent hiccup
Treatment: 110 day, 200 mg/day
Improvement: Tumor size reduced to 1.5 cm, no difficulty in swallow, much less hiccup, no pain, Feeling well by himself

Example 3: Male, 66 yr, Pancreatic cancer 4.5x4.5x3.7 cm
Symptoms: fatigue, dizzy, upper abdominal and liver pain, abnormal high GPT, GOT, and blood sugar
Treatment: 90 day, 200 mg/day
Improvement: Much less pain, GPT , GOT , blood sugar all returned to normal.

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[0042] Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. For example, the present invention comprehends all optical isomers and racemic forms of sapogenin protopanaxadiol and/or sapogenin protopanaxatriol. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. In the claims, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to".

WHAT IS CLAIMED IS:

1. A method of inhibiting the multiplication of cancer cells comprising treating the cells with a therapeutically and synergistically effective combination of:

(a) paclitaxel and sapogenin protopanaxadiol and sapogenin protopanaxatriol;

(b) paclitaxel and sapogenin protopanaxadiol; or

(c) paclitaxel and sapogenin protopanatriol.

2. A method of inhibiting the multiplication of cancer cells comprising treating the cells with a therapeutically and synergistically effective combination of:

(a) mitoxantrone and sapogenin protopanaxadiol and sapogenin protopanaxatriol;

(b) mitoxantrone and sapogenin protopanaxadiol; or

(c) mitoxantrone and sapogenin protopanaxatriol.

3. The method of claim 1 wherein the cancer cells are multi-drug resistant.

4. The method of claim 2 wherein the cancer cells are multi-drug resistant.

5. The method of claim 3 wherein the cancer cells do not express P-glycoprotein.

6. The method of claim 4 wherein the cancer cells do not express P-glycoprotein.

7. The method of claim 1 wherein the cancer cells are selected from the group consisting of prostate cancer cells and breast cancer cells.

8. The method of claim 2 wherein the cancer cells are selected from the group consisting of prostate cancer cells and breast cancer cells.

9. A method of treating a patient having a cancer, comprising treating the patient with a therapeutically and synergistically effective combination of paclitaxel and sapogenin protopanaxadiol or paclitaxel and sapogenin protopanaxatriol, or paclitaxel and sapogenin protopanaxadiol and sapogenin protopanaxatriol..

10. A method of treating a patient having a cancer, comprising treating the patient with a therapeutically and synergistically effective combination of mitoxantrone and sapogenin protopanaxadiol or mitoxantrone and sapogenin protopanaxatriol, or mitoxantrone and sapogenin protopanaxadiol and sapogening protopanaxatriol.

11. The method of claim 9 wherein the cancer is a multi-drug resistant cancer.

12. The method of claim 10 wherein the cancer is a multi-drug resistant cancer.

13. The method of claim 11, wherein the cancer cells do not express P-glycoprotein.

14. The method of claim 12, wherein the cancer cells do not express P-glycoprotein.

15. The method of claim 9 wherein the cancer is selected from the group consisting of prostate cancer and breast cancer.

16. The method of claim 10 wherein the cancer is selected from the group consisting of prostate cancer and breast cancer.

17. The use of a therapeutically effective amount of sapogenin protopanaxadiol or sapogenin protopanaxatriol alone or together in combination with paclitaxel to synergistically inhibit the multiplication of cancer cells.

18. The use of sapogenin 20(R) protopanaxadiol or sapogenin 20(S) protopanaxatriol alone or together to formulate a medicament with paclitaxel for synergistically inhibiting the multiplication of cancer cells.

19. The use of a therapeutically effective amount of sapogenin protopanaxadiol or sapogenin protopanaxatriol alone or together in combination with mitoxantrone to synergistically inhibit the multiplication of cancer cells.

20. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol alone or together to formulate a medicament with mitoxantrone for synergistically inhibiting the multiplication of cancer cells.

21. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol according to claim 17 wherein the cancer cells are multi-drug resistant.

22. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol according to claim 18 wherein the cancer cells are multi-drug resistant.

23. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol according to claim 17 wherein the cancer cells do not express P-glycoprotein.

24. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol according to claim 18 wherein the cancer cells do not express P-glycoprotein.

25. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol according to claim 17 wherein the cancer cells are selected from the group consisting of prostate cancer cells and breast cancer cells.

26. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol according to claim 18 wherein the cancer cells are selected from the group consisting of prostate cancer cells and breast cancer cells.

27. A method of inhibiting the multiplication of multi-drug resistant cancer cells, wherein the cells do not express P-glycoprotein, comprising treating the cells with a therapeutically and synergistically effective combination of sapogenin protopanaxadiol or sapogenin protopanaxatriol alone or together and a chemotherapeutic.

28. The method of claim 27 wherein the chemotherapeutic is paclitaxel.

29. The method of claim 27 wherein the chemotherapeutic is mitoxantrone.

30. The method of claim 27 wherein the chemotherapeutic is cisplatin.

31. The method of claim 27 wherein the multi-drug resistant cancer cells are selected from the group consisting of prostate cancer cells and breast cancer cells.

32. The use of a therapeutically effective amount of sapogenin protopanaxadiol or sapogenin protopanaxatriol alone or together in combination with a chemotherapeutic to inhibit the multiplication of multi-drug resistant cancer cells, wherein the cells do not express P-glycoprotein, and the chemotherapeutic alone is not therapeutically effective to inhibit the multiplication of the cancer cells.

33. The use of sapogenin protopanaxadiol and sapogenin protopanaxatriol singly or together in accordance with claim 32, wherein the chemotherapeutic is paclitaxel.

34. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol singly or together in accordance with claim 32, wherein the chemotherapeutic is mitoxantrone.

35. The use of sapogenin protopanaxadiol or sapogenin protopanaxatriol singly or together in accordance with claim 32, wherein the chemotherapeutic is cisplatin.

36. The use of sapogenin protopanaxadiol and sapogenin protopanaxatriol

according to claim 32 wherein the multi-drug resistant cancer cells are selected from the group consisting of prostate cancer cells and breast cancer cells.

37. A pharmaceutical composition comprising: a pharmaceutically acceptable carrier; and, therapeutically and synergistically effective amounts of sapogenin protopanaxadiol or sapogenin protopanaxatriol singly or in combination and a chemotherapeutic selected from the group consisting of paclitaxel and mitoxantrone.

38. The pharmaceutical composition of claim 37, wherein the chemotherapeutic is paclitaxel.

39. The pharmaceutical composition of claim 37, wherein the chemotherapeutic is mitoxantrone.

40. The pharmaceutical composition of claim 37, wherein the form of the

composition is selected from the group consisting of an orally administrable form, an injectable form, an externally applicable form, a food form, a multi-nutritional product form and an alternative medicine form.

41. The pharmaceutical composition of claim 40, wherein the composition is the orally administrable form and is selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a pill, a liquid, a spirit, a syrup and a limonade.

42. The pharmaceutical composition of claim 40, wherein the composition is the injectable form and is selected from the group consisting of a liquid, a suspension and a solution.

43. The pharmaceutical composition of claim 40, wherein the composition is the externally applicable form and is selected from the group consisting of an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

Example 1. Protopanaxadiol has more potent tumor inhibitory effect than Rh2.

The cytotocixity of protopanaxadiol (PPD), protopanaxatrial (PPT) and Rh2 was compared in B16 melenoma tumor cells. Cells were treated with various concentrations of the compounds and the viability was measured 24 hours post treatment.

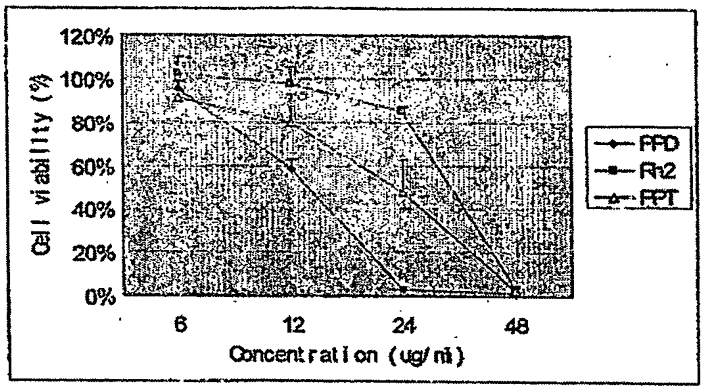

FIG 1

Example 1, Enhanced cytotoxicity of Paclitexal on drug sensitive (MCF7) or drug resistant (MCF7r) human breast cancer cells in the presence of Rh2, Protopanaxadiol (PPD) or Protopanaxatriol (PPT).
Cells were either treated with various concentrations of paclitexal alone or in the presence of 20 μg/ml Rh2, 6 μg/ml PPD, or 6 μg/ml PPT. All the three ginsenoside compounds synergistically enhanced taxol induced cytotoxicity. But PPD is significantly more potent in synergy with taxol. This synergy was most evidenced in drug resistant tumor cells (MCF7r).

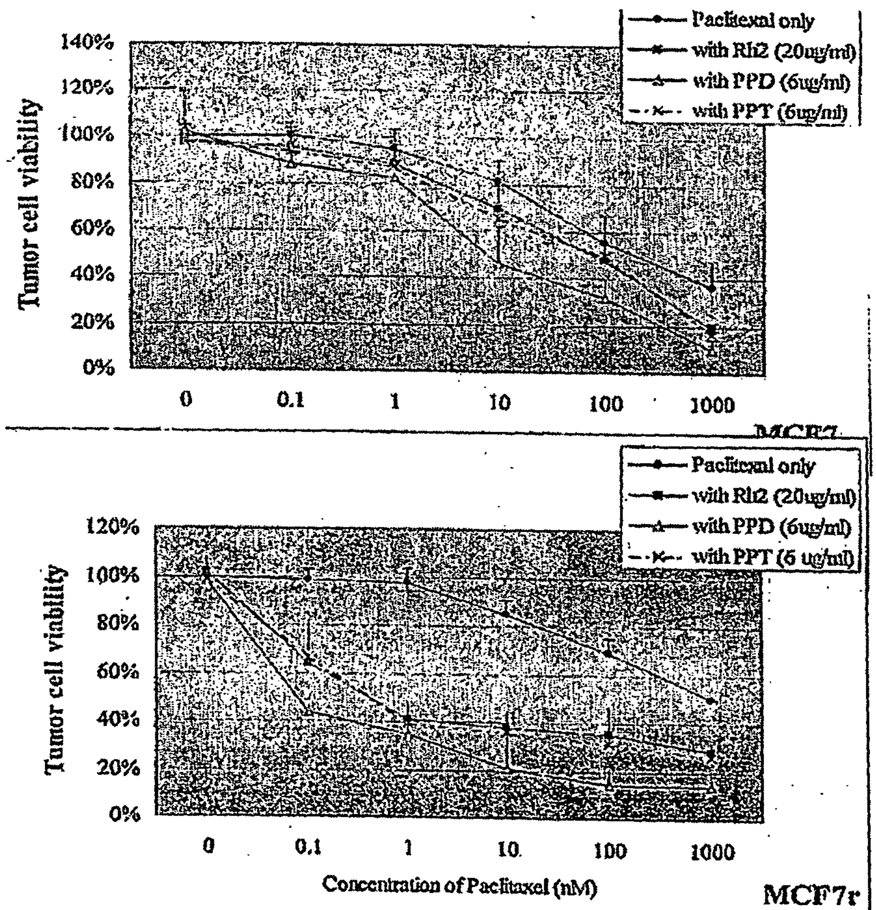

FIG 2a & FIG 2b